Pakistan J. Zool., vol. 52(4), pp 1587-1590, 2020 DOI: https://dx.doi.org/10.17582/journal.pjz/20191019021035

Short Communication

In Silico Profiling of Regulatory MicroRNA Targets in Programmed Cell Death 1 Gene

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Article Information
Received 19 October 2019
Revised 11 December 2019
Accepted 21 December 2019
Available online 22 April 2020

Authors' Contributions
AS, MAN and SAR conceived the idea. AS wrote the manuscript. All other helped in preparation of the manuscript.

Key words
PDCD1, microRNAs, Computational prediction, miRDB and Multiple sequence alignment.

ABSTRACT

MicroRNAs (miRNA) are the novel class of small non-coding RNAs comprising of 17-24 nucleotides that, targets in silencing of one or more genes. Despite their importance as gene regulator, computational strategies are still at its initial stages. Several computer based prediction databases remain the only foundation for rapid identification of putative microRNA target. By utilizing experimentally validated targets, the search of further targets via bioinformatics tools help to predict gene target site. Keeping this in view, a study has been planned to investigate PDCD1 gene regulatory miRNA targets, their sequences and their seed location in human using online server miRDB. We have identified 26 specific miRNAs (hsa-miR-939-3p, hsa-miR-661, hsa-miR-2861, hsa-miR-23b-5p, hsa-miR-23a-5p, hsa-miR-4764-5p, hsa-miR-6734-3p, hsa-miR-4441, hsa-miR-4267, hsa-miR-4456, hsa-miR-7847-3p, hsa-miR-6515-5p, hsa-miR-5692b, hsa-miR-4664-5p, hsa-miR-342-5p, hsa-miR-5692c**,** hsa-miR-3911, hsa-miR-3960, hsa-miR-6852-5p, hsa-miR-4253, hsa-miR-6862-5p, hsa-miR-4296, hsa-miR-4695-5p, hsa-miR-4447, hsa-miR-4532 and hsa-miR-6752-3p) in humans which can target different regions in PDCD1 gene. Multiple sequence alignments were also performed to investigate similarities among mature sequences of these miRNAs. Our data will provide concrete bases for the validation of these miRNAs.

Programmed cell death 1 (PDCD1) also called PD-1 cluster of differentiation 279 (CD279) is a member of B7 superfamily involved in immunomodulation. PDCD1 is an inhibitory cell surface receptor on T-cell, which is involved in the regulation of T-cell function during tolerance and immunity (Xia *et al*., 2018). PDCD1inhibits T-cell effector functions when binds to its ligand PDL1 and PDL2), in an antigen-specific manner. PDCD1 has been studied for cancer, human immunodeficiency virus (HIV), and Alzheimer's diseases. Overexpression of PD1

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0030-9923/2020/0004-1587 $ 9.00/0

on T cells is an indicator of exhaustion of T-cell or T-cell anergy (Syn *et al*., 2017).

MicroRNAs (miRNAs) are the evolutionarily conserved, short (19–25 nucleotides) sequences, single-stranded non-coding RNAs that bind to messenger RNA (mRNA) by a sequence-specific manner and regulate gene expression (Jiang *et al*., 2018) and play important roles in fields of development biology, cancer, infectious diseases and play important roles in various fields of life science. miRNAs hampers translation of target mRNAs into protein and to encourage degradation of mRNA targets which regulate the expression of more than 30% of protein-coding genes at the post transcriptional and translational level (Zhang *et al*., 2018).

The miRDB is an online computational tool for miRNA target prediction and functional annotations. All miRDB targets were predicated by MirTarget used to predict miRNA targets with machine learning methods. miRDB hosts predicted miRNA targets in five species: human, mouse, rat, dog and chicken (Wang, 2008; Chen and Wang, 2019). Thousands of genes in humans are regulated by these miRNAs (Jamieson *et al*., 2012). Despite of their admitted importance, miRNA regulating PDCD1 gene function in human are not well understood. So, the present study was planned to carry out *in silico* prediction of putative miRNA targets in human, with aim to declare early diagnostic markers for the fate of cancer, organ transplant and pregnancy/inflammatory process. *In silico* analyses in the present study for profiling miRNA of PDCD1 gene will be helpful for its correlation with T cell receptor (TCR) signaling.

Materials and methods

List of genes associated with TCR coinhibation identified in humans were collected from literature and publicly available databases. All the relevant publications were identified after searching PubMed with key words immunotolerence, graft transplant, cancer, materofetal or fetomaternal tolerance, Nivolumab *etc*. Out of all the genes obtained after keywords searching, PDCD1 was selected for this computational study as previously described study of Rahman *et al*. (2014).

The sequence of PDCD1 was retrieved from Genbank (NG_012110) (Benson *et al*., 2000). We used miRDB for searching of potential miRNA targets of PDCD1 gene. miRDB is an online computational tool for miRNA target prediction and functional annotations (Wang, 2008). The miRDB (MicroRNA target prediction and functional study data base http://mirdb.org/) was used for potential miRNA target identification in PDCD1 from NCBI Gene ID and predicts miRNA targets in for human.

Results and discussion

By using miRDB bioinformatic database, 26 potential miRNAs targeting PDCD1 gene in human were identified, given in Tables I as previously described study of Rahman *et al*. (2014) who predicted 11 targeting miRNA of GJB3 gene by using miRDB software. The miRDB is the computer based database for identification binding site in selected gene, which will provide strong further basis for experimental validation (Chen and Wang, 2019) of these novel miRNAs in PDCD1. If validated by experiments, these miRNAs might be used as novel biomarkers for regulation of PDCD1 gene.

Tio *et al*. (2018) studied anti-PD-1/PD-L1 immunotherapy in patients with solid organ transplant, HIV or hepatitis B/C infection. They came to know that among 6 patients with solid organ transplant shows graft rejection and including 1 death. Patients with HIV or hepatitis B/C respond well to anti-PD-1/PD-L1 immunotherapeutic treatment without any increased in toxicity.

Guleria and Sayegh (2007) studies that role of PD-1 upon binding with it ligand PD-L1 significantly increase rate of fetal survival due to shifting to Th2 cytokines, which are important for maintenance of successful pregnancy. Compared with the results of current study, PDCD1 may possibly responsible for programmed cell death.

Wang *et al*. (2017) reviewed that binding of PD1/PDL1 has been considered to play an important role in suppressing immune system which leads to invasion of tumor cells rejection of organ transplant and failure of fetomaternal tolerance. Thus, this study will provide the millstones for future researches to look on putative miRNA of PDCD1 gene.

The miR-661 has been well known to be involved in development of cancer in human beings (Wang *et al*., 2017). The hsa-miR-661 has helps the proliferation of the human cancerous cell line, this suggested that it may be a potential in therapy of lung cancer (Lu *et al*., 2019). miR-661 prevents the proliferation of human glioma cells, and their invasion and migration by targeting Telmerase reverse transcriptas TERT/hTERT) (Li *et al*., 2015). Another previous study reported that miR-661 can promotes the ovarian cancer cells by directing targeting the inositol polyphosphate-5-phosphate J (INPP5j) gene (Zhu *et al*., 2015).

Apoptosis, a form of program cell death has been studied in many degenerative diseases (Cheng *et al*. 2011). The downregulation of miR-2861 may prevents the apoptosis of endometriotic tissue through the regulation of the MMp2 and STAT3. So, miR-2861 might be a potential therapeutic biomarker in endometriosis (Yu *et al*., 2019). The previous study identified the miR-2861 as a regulator of cardiomyocyte necrosis and suggest potential therapeutic biomarker in cardiac disease (Weng *et al*., 2016). miR-6734 arrested the cellular growth and causes

Table I.- miRNA targeting PDCD1 in human and list of 26 miRNA predicated by online tool miRDB in the sequence of Programmed cell death 1 (PDCD1) gene. Gene 5133 is predicted to be targeted by 26 miRNAs in miRDB.

S. No.	Precursor miRNA		Mature miRNA				Target Gene specifications		
	miRBase ID	Precursor name	miRBase ID	miRNA name	Sequence	Length	Genomic location	Seed sequence	Seed location
1	MI0005761	hsa-mir-939	MIMAT0022939	hsa-miR-939-3p	5' - CCCUGGGCCUCUGCUCCCCAG - 3'	21	chr8:144394149-144394230 (-)	GCCCAGG	201, 627
2	MI0003669	hsa-mir-661	MIMAT0003324	hsa-miR-661	5' - UGCCUGGGUCUCUGGCCUGCGCGU-3'	24	chr8:143945191-143945279 (-)	CCCAGGC	202, 628
3	MI0013006	hsa-mir-2861	MIMAT0013802	hsa-miR-2861	5' - GGGGCCUGGCGGUGGGCGG - 3'	19	chr9:127785918-127786007 (+)	CAGGCCC	642, 704, 820
4	MI0000439	hsa-mir-23b	MIMAT0004587	hsa-miR-23b-5p	5' - UGGGUUCCUGGCAUGCUGAUUU - 3'	22	chr9:95085208-95085304 (+)	GGAACCC	913
5	MI0000079	hsa-mir-23a	MIMAT0004496	hsa-miR-23a-5p	5' - GGGGUUCCUGGGGAUGGGAUUU - 3'	22	hr19:13836587-13836659 (-)	GGAACCC	913
6	MI0017405	hsa-mir-4764	MIMAT0019914	hsa-miR-4764-5p	5' - UGGAUGUGGAAGGAGUUAUCU - 3'	21	chr22:33436582-33436669 (-)	CA CATCC	1029
7	MI0022579	hsa-mir-6734	MIMAT0027370	hsa-miR-6734-3p	5' - CCCUUCCCUCACUCUUCUCUCAG - 3'	23	chr1:43364648-43364715 (-)	GGGAAGG	563, 971
8	MI0016784	hsa-mir-4441	MIMAT0018959	hsa-miR-4441	5' - ACAGGGAGGAGAUUGUA - 3'	17	chr2:239085827-239085926 (-)	CTCCCTG	292, 537
9	MI0015871	hsa-mir-4267	MIMAT0016893	hsa-miR-4267	5' - UCCAGCUCGGUGGCAC - 3'	16	chr2:110069961-110070042 (-)	GAGCTGG	689
10	MI0016802	hsa-mir-4456	MIMAT0018978	hsa-miR-4456	5' - CCUGGUGGCUUCCUUUU - 3'	17	chr5:535840-535882 (-)	CCACCAG	14,955, 1066
11	MI0025517	hsa-mir-7847	MIMAT0030422	hsa-miR-7847-3p	5' - CGUGGAGGACGAGGAGGAGGC - 3'	21	chr11:1880045-1880147 (+)	CCTCCAC	33, 610
12	MI0022227	hsa-mir-6515	MIMAT0025486	hsa-miR-6515-5p	5' - UUGGAGGGUGUGGAAGACAUC - 3'	21	chr19:12940484-12940540 (+)	CCCTCCA	32, 609
13	MI0019311	hsa-mir-5692b	MIMAT0022497	hsa-miR-5692b	5' - AAUAAUAUCACAGUAGGUGU - 3'	20	chr21:42950928-42951014 (-)	ATATTAT	1139
14	MI0017294	hsa-mir-4664	MIMAT0019737	hsa-miR-4664-5p	5' - UGGGGUGCCCACUCCGCAAGUU - 3'	22	chr8:143733083-143733153 (-)	GCACCCC	1043
15	MI0000805	hsa-mir-342	MIMAT0004694	hsa-miR-342-5p	5' - AGGGGUGCUAUCUGUGAUUGA - 3'	21	chr14:100109655-100109753 (+)	GCACCCC	1043
16	MI0019288	hsa-mir-5692c-1	MIMAT0022476	hsa-miR-5692c	5' - AAUAAUAUCACAGUAGGUGUAC - 3'	22	chr5:135802985-135803075 (-)	ATATTAT	1139
17	MI0016415	hsa-mir-3911	MIMAT0018185	hsa-miR-3911	5' - UGUGUGGAUCCUGGAGGAGGCA - 3'	22	chr9:127690687-127690795 (-)	TCCACAC	1033
18	MI0016964	hsa-mir-3960	MIMAT0019337	hsa-miR-3960	5' - GGCGGCGGCGGAGGCGGGGG - 3'	20	chr9:127785833-127785923 (+)	CCGCCGC	732
19	MI0022698	hsa-mir-6852	MIMAT0027604	hsa-miR-6852-5p	5' - CCCUGGGGUUCUGAGGACAUG - 3'	21	chr9:35710676-35710741 (-)	CCCCAGG	521
20	MI0015860	hsa-mir-4253	MIMAT0016882	hsa-miR-4253	5' - AGGGCAUGUCCAGGGGGU - 3'	18	chr1:22863159-22863226 (-)	CATGCCC	624
21	MI0022709	hsa-mir-6862-1	MIMAT0027625	hsa-miR-6862-5p	5' - CGGGCAUGCUGGGAGAGACUUU - 3'	22	chr16:28390982-28391051 (-)	CATGCCC	624
22	MI0015823	hsa-mir-4296	MIMAT0016845	hsa-miR-4296	5' - AUGUGGGCUCAGGCUCA - 3'	17	chr10:125032783-125032870 (-)	G CCCACA	190
23	MI0017328	hsa-mir-4695	MIMAT0019788	hsa-miR-4695-5p	5' - CAGGAGGCAGUGGGCGAGCAGG - 3'	22	chr1:18883202-18883275 (-)	GCCTCCT	382, 724
24	MI0016790	hsa-mir-4447	MIMAT0018966	hsa-miR-4447	5' - GGUGGGGGCUGUUGUUU - 3'	17	chr3:116850277-116850367 (-)	CCCCC AC	1046
25	MI0016899	hsa-mir-4532	MIMAT0019071	hsa-miR-4532	5' - CCCCGGGGAGCCCGGCG - 3'	17	chr20:57895394-57895444 (+)	CC CCGGG	989
26	MI0022597	hsa-mir-6752	MIMAT0027405	hsa-miR-6752-3p	5' - UCCCUGCCCCCAUACUCCCAG - 3'	21	chr11:67490245-67490315 (+)	GGCAGGG	77, 594, 656, 801, 1059

the apoptosis of colon cancer cells by up-regulating the p-21 genes, which is suggesting the its key role in cancer cell survival and growth.

The miR-342 regulates the lumen formation of mammary gland morphogenesis (Weng *et al*., 2016). The Polymerase chain reaction (PCR) is a sensitive of molecular identification (Shaukat *et al*., 2019). Ding *et al*. (2016) has predicted that differentially expressed miRNAs in type 2 diabetes mellitus (T2DM) including hsa-miR-3960 by real time PCR. Their study might be helpful in subsequent experiments in T2DM.

Details of names of candidate miRNAs, their sequences, order of nucleotides, seed sequences and location target score and protein size of target gene has been shown in Table I, multiple sequence alignment, phylogenetic tree analysis of all the 26 miRNAs and purines and pyrimidines alignment of 26 miRNAs are shown in Supplementary Figure 1. All the miRNA targeting PDCD1 on miRDB were displayed in Tables I and Supplementary Figure 1.

Conclusion

The miRNAs are the novel class of small non-coding RNAs that targets in silencing of one or more genes. Keeping in view of importance of computer based prediction databases miRDB has been used in our study to mine the miRNAs targeting PDCD1 gene in human beings. We have identified 26 specific miRNAs in humans which can target different regions in PDCD1 gene. Our data will provide concrete bases for the validation of these miRNAs in silencing of PDCD1 gene.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20191019021035

Statement of conflict of interest

Authors have declared no conflict of interest.

References

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Rapp, B.A. and Wheeler, D.L., 2000. *Nucl. Acids Res.*, **28**: 15-18.

Cheng, X.S., Li, M.S., Du, J., Jiang, Q.Y., Wang, L., Yan, S.Y., Yu, D.M. and Deng, J.B., 2011. *Anatom. Histol. Embryol.*, **40**: 21-27. https://doi.org/10.1111/j.1439-0264.2010.01033.x

Chen, Y. and Wang, X., 2019. *Nucl. Acids Res.,* **48**: D127-D131. https://doi.org/10.1093/nar/gkz757

Chicurel, M., 2002. *Nature*, **419**: 751-758.

Ding, L., Ai, D., Wu, R., Zhang, T., Jing, L., Lu, J. and Zhong, L., 2016. *Biosci. Biotechnol. Biochem.*, **80**:461–465.

Guleria, I. and Sayegh, M.H., 2007. *J. Immunol.*, **178**: 3345-3351.

Jamieson, N.B., Morran, D.C., Morton, J.P., Ali, A., Dickson, E.J., Carter, C.R., Sansom, O.J., Evans, T.J., McKay, C.J. and Oien, K.A., 2012. *Clin. Cancer Res.*, **18**: 534-545. https://doi.org/10.1158/1078-0432.CCR-11-0679

Jiang, K., Guo, S., Zhang, T., Yang, Y., Zhao, G., Shaukat, A., Wu, H. and Deng, G., 2018. *Front. Pharmacol.*, **9**: 142. https://doi.org/10.3389/fphar.2018.00142

Li, Z., Liu, Y.H., Diao, H.Y., Ma, J. and Yao, Y.L., 2015. *Biochem. biophys. Res. Commun.*, **468**: 870-876. https://doi.org/10.1016/j.bbrc.2015.11.046

Lu, J., Gu, X., Liu, F., Rui, Z., Liu, M. and Zhao, L., 2019. *Oncol. Rep.*, **41**: 2987-2996.

Rahman, K., Shah, A.A., Khan, M.H. and Ullah, S., 2014. *Glob. J. Biotechnol. Biochem.,* **9**: 41-49.

Shaukat A., Khalid M., Irfan S., Tauseef u.R., Muhammad A.N., Ashar M., Muhammad I. S., Zia-ud-Din Sindhu, Shahid A.R., Mubashar H., Saqib U., Muhammad A.J., Rao Z.A. and Anas S.Q., 2019. *Pakistan J. Zool.,* **51**: 2023-2032.

Syn, N.L., Teng, M.W., Mok, T.S. and Soo, R.A., 2017. *Lancet Oncol.*, **18**: e731-e741. https://doi.org/10.1016/S1470-2045(17)30607-1

Tio, M., Rai, R., Ezeoke, O.M., McQuade, J.L., Zimmer, L., Khoo, C., Park, J.J., Spain, L., Turajlic, S., Ardolino, L. and Yip, D., 2018. *Eur. J. Cancer*, **104**: 137-144. https://doi.org/10.1016/j.ejca.2018.09.017

Wang, X., 2008. *RNA*, **14**: 1012-1017.

Wang, K., Long, B., Li, N., Li, L., Liu, C.Y., Dong, Y.H., Gao, J.N., Zhou, L.Y., Wang, C.Q. and Li, P.F., 2016. *Free Rad. Biol. Med.*, **91**: 58-67.

Wang, Y., Li, Y., Wu, B., Shi, C. and Li, C., 2017. *Mol. Med. Rep*., **16**: 2113-2120. https://doi.org/10.3892/mmr.2017.6827

Weng, C., Nguyen, T. and Shively, J.E., 2016. *J. biol. Chem.*, **291**: 16777-16786. https://doi.org/10.1074/jbc.M115.710152

Xia, Q., Wei, L., Zhang, Y., Sheng, J., Wu, W. and Zhang, Y., 2018. *Mediators Inflammat*., **2018**: Article ID 1632902. https://doi.org/10.1155/2018/1632902

Yu, H., Zhong, Q., Xia, Y., Li, E., Wang, S. and Ren, R., 2019. *Die Pharmazie-An Int. J. Pharmaceut. Sci.*, **74**: 243-249.

Zhang, T., Jiang, K., Zhu, X., Zhao, G., Wu, H., Deng, G. and Qiu, C., 2018. *Int. J. biol. Sci.*, **14**: 622. https://doi.org/10.7150/ijbs.24223

Zhu, T., Yuan, J., Wang, Y., Gong, C., Xie, Y. and Li, H., 2015. *Biomed. Pharmacother.*, **75**: 123-128.